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Localisation and Formation of the Alcaloid in Cinchona succirubra and Ledgeriana

by

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On the 29th. of September 1896 the undersigned received an instruction charging him with „botanical-physiological investigations—microchemical and experimental—concerning the ways and conditions under which the alcaloids of Cinchona plants are formed, transported, accumulate and increase or decrease.

Since then the following articles, besides short abstracts in the quarterly and annual reports of the Cinchona Gardens, have appeared:

1. Die Localisationen des Alkaloids in Cinchona calisaya Ledgeriana und in Cinchona succirubra. (Botanisches Centralblatt. Bd. L XXI. 1897.)
2. Een en ander over Reservevoedsel. (Archief voor de kinacultuur № 4. 1898.)
3. De localisatie van het alcaloid in Cinchona calisaya Ledgeriana en in Cinchona succirubra (with 36 figures in the text and an atlas of 20 coloured plates). Batavia, 's Landsdrukkerij, 1898. (1)

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(1) The dutch text with plates can be had from the firm G. Kolff & Co, Booksellers, Batavia and the Hague.

4. Physiologische proeven genomen met Cinchona succírubra. Ie Stuk. Waar wordt het alcaloid gevormd? Batavia-'s Gravenhage G. Kolff & Co. 1899.

It was thought desirable to give a resumé of these Dutch articles in a more generally understood language, it is therefore that I beg leave to offer them to the public in the following pages.

J. P. Lotsy.

Tjibodas. Oct. 1899.

Part. I. The localisation of the alcaloid.

A. Method.

The method here followed is an adaptation of the general methods for the localisation of alcaloids described by L. Errera and his pupils. (1)

Several reagents precipitate the alcaloids in Cinchona cells but none of them is typical for the alcaloids exclusively, albuminous substances giving very similar reactions. Bij good fortune there is a way however to distinguish them neatly, it is based on the solubility of alcaloids and the insolubility of albuminous substances in acid alcohol.

A precipitate caused by the alcaloid reagents in the Cinchona cells consequently indicates the presence of alcaloid or that of an albuminous substance. To decide this, we make two sections of the organ under investigation; the one is put at once under the alcaloid treatment, the other is first extracted by means of acid alcohol. The one section consequently contains the alcaloid in normal quantity, the other is entirely free from it.

Treating both with the same reagent, in the same concentration and obtaining a precipitate in each we may safely conclude this precipitate to be due to the presence of an albuminous substance. If on the other hand no precipitate appears in the extracted section, while a profuse one is formed in the non-extracted one, it is caused by an alcaloid.

B. Choice made out of the different reagents.

To find the most suitable reagents, that is to find those which gave the most conspicuous precipitates a large number of reagents was tested macrochemically on solutions,

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(1) Errera, Maistriau et Clautriau. Premières recherches sur la localisation et la signification des alcaloides dans les plantes,. Bruxelles 1887.—

largely diluted, of the rough Cinchona-alcaloids. These testings were repeated with the same solutions to which some drops of tannic acid had previously been added. This was done because preliminary experiments had shown the presence of both alcaloid and tannic acid in the same cell; it was consequently of prime importance to study the influence of the tannins on the reagents used. A beautifully prepaired quantity of the Rough-alcaloid was obtained by the kindness of mr. P. van Leersum, the functionating Director of the Cinchona Gardens.This was dissolved in slightly acidulated water and subsequently used for microchemical tests. A drop of the solution was mixed on the slide with a drop of one of the alcaloid-reagents, examined under the microscope and the precipitate drawn by means of the camera lucida, both with the mirror of the microscope turned on and off.

The two rows to the left of Plates I, II and III of the Dutch edition show these drawings in the natural colors. They picture the precipitates obtained by ammonia liquida, chromic acid, congo-red, eosine, chloride of gold, iodine in watery solution, iodine dissolved in a solution of iodide of potassium, double iodide of potassium and mercury, bichromate of potassium, ferricyanide of potassium, ferrocyanide of potassium, caustic potash, permanganate of potassium, molybdate of ammonia, bicarbonate of sodium, monocarbonate of sodium, phospho-molybdic acid, picric acid, chloride of platinum, salycilic sodium and by corosive sublimate.

These reagents were consequently *all* used to precipitate the alcaloid in the cells of the leafstalk of Cinchona Ledgeriana. As is seen from the two rows of pictures to the right of Plates I, II and III of the Dutch edition this succeeded with all. *Consequently the presence of the alcaloid in the cells of the leafstalk of C. C. Ledgeriana has been de-*

monstrated bij the aid of some twenty reagents.

It was demonstrated at the same time that alcaloid and tannic acid are frequently found in the same cell most probably the alcaloid is present as a tannic acid salt.

In many cases it was shown simultaneously that the alcaloid is dissolved in the cellsap.

Yet, these reagents are not all of equal value for our purpose. Many become unclear owing to the presence of tannins, others penetrate badly into the cells, others again have a destroying influence on the cellwalls or on the protoplasm, thus causing the alcaloid to escape from the cells etc.

The reagent best adopted to our needs proved to be a solution of iodine in iodide of potassium.

It was this solution that was mostly used but *when the least bit of doubt was caused by the aspect of the precipitate thus obtained, or if the absence of a precipitate made us wonder, other reagents where always used to put the result obtained by the iodine solution to the test.*

C. Concentration and mode of using the Iodine—solution (1)

The most practical way of making the Iodine-solution is thus: In a certain quantity of water a rather large quantity of iodide of potassium is dissolved, how large a quantity is of little importance provided it be not too little. It is however of *prime* importance that this solution be consequently *absolutely saturated* with Iodine as free Iodide of potassium dissolves the alcaloid.

This solution is kept in stock.

Shortly before using, so much of it is mixed with water that this solution, poured into a watchglass is of the colour of vermouth. To obtain comparable results a solution of the

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(1) So called for shortness' sake.

same colour was always used. Subsequently the following mode of proceeding was ressorted to.

Of the organ under investigation sections were made (by means of a razor) which may not be thinner than one whole layer of cells, as the alcaloids escape from cells opened by the knife. These sections are submersed and for a moment gently moved in water to remove the alcaloid in the rests of the opened cells. Meanwhile three watchglasses containing the Iodine-solution, above mentioned, are prepaired. The sections are now put in the first watchglass and gently stirred by means of a glass rod. If alcaloid in a somewhat considerable quantity be present a cloud will appear in the otherwise clear Iodine-solution. This cloud consists of alcaloid, gummy substances, starch etc. escaping from the opened cells. As soon as this cloud is observed the section is removed to the second watchglass, stirred gently again and as soon as a cloud has formed here also, removed to the third watchglass. Generally no cloud will be formed in this glass, if unexpectedly this might yet happen the section is removed to the fourth and if necessary to the fifth watchglass.

If this mode of proceeding is *not* followed the substances above mentioned form a cake on the section which makes it unsuitable for observation under the microscope.

In the last watchglass the sections remain for about fifteen minutes; a longer sojourn in the solution does no harm if one bears in mind that a long submersion causes the alcaloid to flow together to oily drops, which finally may form one large drop in the cell.

Finally the section is washed with water for a moment and mounted in water also. Sometimes it was deemed advisable to submerse the section for a moment in a solution. containing 2,5 cc. of concentrated sulfuric acid, 25 cc. alcohol of 96°/₀ and 72,5 cc. of water; when the quantity

of alcaloids in the cells is not *too* small no harm is caused by this, while it is very advantageous in as much as the precipitate after this treatment becomes much less soluble in glycerine.

Sections so treated can subsequently be mounted and examined in glycerine which on account of the clearifying properties of the glycerine is of no small advantage with necessarily rather thick sections.

If very little alcaloid be present in the cells the mode of proceeding just described, is unadvisable. In that case it is much better to mount the section after being well washed in water and subsequently cause a drop of the stocksolution of iodine, put at the border of the cover-slip, to diffuse into the mounting fluid. In this way one can see the precipitate originate in the cells. With bicarbonate of potassium aud such like reagents which have a plasmolysing influence the same mode of proceeding is highly advisable. Bij means of them one first observes the contraction of the vacuole and the cellsap is seen as a clear globe. The reagent has not yet penetrated through the wall of the vacuole; now it does and behold the appearance of the precipitate as if the cell were touched bij the sorcerer's rod. A fine instance of this has been pictured in fig 64 Pl III. (1)

Wherever cells greatly stretched in longitudinal direction are met with another diffculty occurs. In those cases, for example in the leafstalk, all cells are opened by a cross-section, the alcaloid escapes; or if one makes a section with unopened cells it is so thick that nothing can be distinguished. It is of course possible to examine a longitudinal section of such an organ, but frequently

(1) Wherever now and hereafter reference is made to figures and plates the atlas of the Dutch text is meant.

a cross-section would give much plainer results and one regrets to be unable to obtain one.

In such cases I frequently obtained very good results by cutting across the leaf stalk under a diluted Iodine-solution thus causing the leaf by means of its transpiration to suck in the Iodine-solution. Yes, based on Strasburgers investigation concerning the mounting of poisonous solutions in trees, I even made large branches suck in the solutions, thus obtaining very good results indeed.

If one subsequently makes cross-sections of such an organ it does not matter wether the cells are opened or not, the precipitate remains at the spot where it was formed provided one's razor be sharp enough.

A sharp razor cuts through the precipitate and leaves it in position, a blunt one tears it out of the cells.

D. Choice of Cinchona species.

At first Cinchona Calisya Ledgeriana was used exclusively, later it was fonnd that many points were much clearer in C. succirubra consequently that species was investigated also and the results compared.

E. The localisation of the alcaloid.

A. The leaf.

1. *The epidermis.*

At no time does the epidermis contain any alcaloid, neither in the young nor in the old leaf. It is true one obtains occasionally a reaction which might lead one to accept the presence of traces of alcaloid, but even then it occurs only in a few of the epidermal cells. Anyhow, if present at all, it is of no significance whatever, neither do the hairs or closing cells of the stomata contain any alcaloid.

2. *The Hypoderm.*

At the upperside of Cinchona leaves a large—celled, colorless subepidermal cell-sheath is observed, it is always present in C. Ledgeriana; in C. succirubra it may be absent over a larger or smaller distance.

As long as the chlorophyll has not yet appeared in the very young leaf, this subepidermal layer can not be distinguished from the other cells anatomatically. But even then it frequently is conspicuous by its large amount of alcaloid. This is also the case in adult leaves; even where it takes trouble to discover the alcaloid in the green cells of a leaf, that leaf will show it plainly in its hypoderm.

This can be seen very nicely on a tangential section of the upperside of the leaf blade. The finest sections are those where the knife separated exactly the hypoderm from the palisade-parenchyma. Such a section is absolutely colorless and by the action on it of picrid acid the picture becomes very plain indeed. If one regards such a section from above one sees (through the clear layer of epidermal cells with undulated walls) the precipitate in the large polygonal straight-walled hypodermal cells. Those hypodermal cells which cover the leaf-veins are stretched in the direction of these veins. Apparently they contain even more alcaloid than the other subepidermal cells. The appearance of the hypodermal cells after the alcaloid has been precipitated in them may be judged off by contemplation of fig. 90, 91 Pl. V. fig 96 Pl. VI. fig 98, 99 Pl.VII.

3. *The Mesophyll.*

Neither C. C. Ledgeriana, nor C. succirubra, contains any alcaloid in the very young parenchyma; (compare x′ in fig 89 Pl. V.) on a somewhat older stage (comp. x. fig-87 Pl. IV.) the case alters, it appears gradually and in this stage all green cells contain it in large quantity (c. fig. 90. Pl. V).

In adult leaves of C. succirubra alcaloid can be demonstrated to be present (at certain times) in all mesophyll cells but always in less quantity than in the younger leaves. Frequently, though not always, it can be demonstrated that the mesophyll cells near the vascular bundles contain more alcaloid than the other mesophyll cells; one sees this occasionally very beautifully on sections paralell to the leaf surface having passed just above a vascular bundle. One then sees mesophyll-cells especially rich in alcaloid in a direction corresponding to the direction taken by the vascular-bundle.

On such happy sections one can judge of the direction originally taken by the removed vascular-bundle, by the large amount of alcaloid present in these mesophyll cells. Apparently the cells of the palissade-parenchyma contain most of the alcaloid and frequently it is seen accumulated at the sides of these cells bordering on the hypoderm (c. fig 99 Pl. VII).

Etiolated leaves also contain alçaloid in all parenchyma cells frequently more than green leaves do (c. f. 100. Pl. VII).

These leaves had been developped under cover of a large box from resting buds of an old Cinchona-trunk. Following the method of TREUB, small holes were made in them by means of a hairbrush and the whole submersed in the iodine solution. The brown precipitate of the alcaloid is subsequently seen around these holes; (c. fig 108. Pl.VIII) other leaves treated exactly in the same way, but previously having been extracted with alcohol do *not* show this brown precipitate, thus proving it to be due not to albuminous substances but to alcaloid (c. fig 109. Pl. VIII).

Is it legitimate to conclude from these experiments that the alcaloid can be formed in the dark? By no means; it can easily have been substracted by the leaves from the large quantity present in the bark, which bark originated while the tree grew in the light.

In normal green adult leaves of C. S. the alcaloid has easily been demonstrated by means of the iodine potassium-solution (fig. 99 Pl. VII. fig. 107 Pl. VIII) picric acid, platinum chloride, corrosive sublimate, double iodine of potassium and mercury and bicarbornate of sodium.

The same thing holds true for leaves of Cinchona Ledgeriana but the co-occurrence of a dextrine and of an albuminous substance make it *exceedingly* difficult to demonstrate its presence. For particulars I must refer to the dutch text. (fig. 102 Pl. VIII shows the alcaloids in the leaf of C. Ledgeriana, fig. 103 the dextrine albuminous substance, fig. 104 also, fig. 105 and 106 show the yellow and brown precipitate by molybdaenic ammonia in the epidermal cells, the colorless alcaloid-precipitate in the hypoderm).

4. *Midrib, veins and vascular-bundles of the leaf.*

The xylem-part contains no alcaloid, the colorless, reduced sievetubes „Uebergangs-Zellen" of the finest veins neither, nor does the mesophyllsheath of these finest veins.

The mesophyll cells bordering on the mesophyll sheath, on the other hand, contain much alcaloid.

In somewhat thicker veins we find between the xylem and the „Uebergangszellen" some layers of longitudinally stretched cells forming an approach to a phloëm part but in which sievetubes can not yet be distinguished. These cells contain no alcaloid. In the mesophyll sheath of such somewhat thicker veins alcaloid could be demonstrated (fig 115 Pl. IX).

In somewhat larger veins sievetubes are present, these contain no alcaloid nor do their conducting cells. The vascular bundle of these thicker veins is not surrounded by the mesophyll directly but enclosed in a colorless parenchyma with collenchyma at the periphery. This tissue protrudes at both sides of the leaf and it is this tissue which

we see with the naked eye and call veins. In the center of this tissue the vascular bundle or bundles are situated, a mesophyll-sheath is absent of course, its function and position is taken by the starch sheath.

The epidermis cells of the leaf-veins contain no alcaloid, all parenchyma and collenchyma-cells do. Wether the celllayer between phloëm and xylem in these thin veins contains alcaloid or not is a question I do'nt dare to decide; I never saw it there but it is so difficult to obtain sections of these thin veins showing this layer clearly and intact that I have not been able to make a large number of observations.

Mesophyll-cells or cells belonging to the veins, containing oxalic acid, never contain alcaloid.

5. *The leafstalk.*

The leafstalk consists of a parenchyma, in the center of which a ring of vascular-bundles showing some thickning growth is seen.

Inside of this ring we observe some other vascular bundles more or less irregularly distributed, while outside of the vascular bundle at the upper side of the leafstalk usually a couple of small bundles are met with, one to the right and one to the left (c. fig. 110 Pl. IX). The outside of the leafstalk contains the epidermis and hairs; they contain no alcaloid. Under the epidermis we find several layers of collenchyma, which contain alcaloids (c. fig. 110, 111 Pl. IX). Proceeding towards the center we first meet with several layers of large parenchyma-cells containing a large quantity of alcaloids (fig. 110, 112. Pl IX.), subsequently with the starch-sheath with no alcaloids and finally with the with pericycle containing alcaloid.

The parenchyma situated between phloëm bundles contains alcaloids. In the large cells like C. fig. 113 Pl. IX and fig.

116 Pl. X it is always met with, in the small ones it may be absent or present.

The cambium situated between xylem and phloëm usually contains no alcaloid; yet it is met with occasionally (c. fig. 116. Pl X). It is most frequently met in the cambiumcells forming the prolongation of the medullary rays.

In all parenchymacells between xylem and phloem, be they cambiumcells, cells of the pericyle or parenchyma of the phloëm-part alcaloid may be met with; it is never found in the sievetubes nor in the conducting cells and this is a point of some interest.

The medullary rays of the xylem-part can contain alcaloid but frequently do not (fig. 116 Pl. X is a spot chosen for its large amount of alcaloids).

The pith-parenchyma contains alcaloid also; cells which contain oxalic acid possess no alcaloid. (fig. 110 Pl. IX). To get good crosssections with the alcaloid in position the leaves were forced to suck in the iodine solution as described before. In using this method one must not loose sight of the fact that the absence of a precipitate in some particular cell does not yet prove that there was no alcaloid in that cell; it being possible that the iodine solution did not enter it. If the cell under consideration contains starch the entrance or non entrance can be easily determined by the blue color of the starch or the absense of that tint. If the starch is not stained blue and consequently the iodine-solution has not entered, longitudinal sections must be made.

Fig. 110. Pl. IX is made after a crosssection obtained from a leafstalk, which previously had sucked in the iodine solution. Very conspicuous in this section is the halfmoon shape of the precipitate and the fact that inside of the vascular-bundle ring this moon is found at the *outer* cellwall, outside of the bundle-ring at the *inner*

cellwall. This phenomenon is explained by the fact that the iodine solution was transported through the vascular bundles and from there diffused into the surrounding tissues.

The solution consequently reaches at the cells inside of the bundle-ring the outer wall first, at those outside the inner wall first and precipitates the alcaloid at the point of entrance. This is shown plainly in fig. 111 and 112 Pl. IX. The small bundles at the upper side of the leaf-stalk conduct the water also as is seen from the fact that the half-moonshaped precipitate is formed at the cellwalls turned towards them (c. fig. 110 pl. IX). In the so called „Gummiharzschläuche" of de Bary I have been unable to demonstrate the alcaloid, yet there may be some in it, as the large quantities of tannins and rosin in their interior make microchemistry unreliable here.

6. *The budscales.*

The budscales of Cinchona are peculiar on account of the special glands present on their interior side which glands secrete a rosinous substance. They consist of an internal bundle of elongated cells covered by a layer of cells which reminds us strongly of animal epithelium (c. fig. 89 Pl. V.) These epithelial cells contain no alcaloid, the central cells do. Considerable quantities of alcaloid are met with in all parenchyma cells of the budscales, the epiderm and hairs contain no alcaloid (c. fig. 89. Pl. V.) apparently the plant is not very economical as far as its alcaloid is concerned as the leafscales, which have been dropped contain alcaloid yet. (c. fig. 92. Pl V.)

B. The stem.

1. *The primary stem-tissues.*

As long as no trace of differentiation is apparent in the growing point (c. fig. 89 Pl. V.) no alcaloid is found there. As

soon as the vascular bundle-initials become differentiated alcaloid is met with everywhere except in these initials and in the epiderm. On a somewhat older stage like fig. 93 Pl. VI we see, using a magnifying power of about 17 times, no alcaloid in the primary vascular bundles (1) while lots of alcaloid is seen in the primary bark (not in its epiderm however) and it is further observed that the quantity of alcaloid in the pith decreases in a direction from the periphery towards the center. Later on, when the pith dies the alcaloid disappears from it.

For convenience's sake we will treat separately of the tissues inside of the starchsheath and of the starchsheath, and of the tissues outside of it combined. This cutting up of the stem into two parts is perfectly legitimate as the starch-sheath is the innermost layer of the primary bark.

1a. *Tissues inside of the starch-sheath.*

Proceeding from the exterior towards the interior we can distinguish the pericycle consisting of one or two layers of cells containing alcaloid. Then we meet with a ring of vascular bundles in which the cambium begins to divide at a very early stage. Between the different vascular bundles small bands of parenchyma, the pithrays, are seen, these contain alcaloid both in the part between the phloëm-bundles and that situated between the xylem-bundles. In the region of the cambium they contain no alcaloid, nor does the intervascular cambium (c. fig. 117 Pl X).

The cambium contains no alcaloid wherever it may be situated (c. fig. 120, 121 Pl. XI, fig. 124, Pl. XII, fig. 125 Pl. XII); as soon as the parenchyma—cells formed by the action of the cambium, enter on a period of rest they do contain alcaloid however.

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(1) In some of its parts there *is* alcaloid, but too little to be observed at this slight enlargement. See further down.

A tangential section. (fig. 118 Pl. X) shows the presence of some alcaloid in the vasal parenchyma, while a longitudinal section fig. 119. Pl. X) shows large quantities of it in the cribral-parenchyma.

1b. *The Starchsheath and the tissues outside of it.*

This is synonymous with primary bark. The inner layer of it, the starchsheath, contains no alcaloid (c. fig. 117 Pl. X. fig. 120, 122 Pl. XI) all other cells with the exception of those which contain oxalic acid, the gummiharzschläuche, and the epiderm, do.

2. *The secondary tissues.*

2a. *The Wood.*

The cambium generally contains no alcaloid but sometimes one meets with it there. It seems to me that it is only found when the cambuim is inactive, yet I am not quite sure of this.

As soon as the cambium cells have entered on a period of comparative inactivity preparing themselves so to speak for the coming changes in their function they contain alcaloid no matter wether later on they will become vessels, woodfibres or whatever else.

The adult woodvessels never contain any alcaloid, the adult woody fibres very rarely (c. fig. 128 pl. XIII at x).

The cells of the medullary rays and their plate-like prolongations do contain alcaloid and starch as do the woodparenchyma-cells even in the eldest layers of branches of a diameter of 1 decimeter (older ones were not investigated in this respect) most alcaloid is found in the cells of the medullary rays less in those of their prolongations and but little in the wood-parenchyma (c. fig. 126, 127, Pl. XII and fig. 128 Pl. XIII).

2b. *The secondary bark.*

(compare fig. 129 Pl. XIII, fig. 130. 131. Pl. XIV, 132, 133, 134, 135. Pl. XV, 136, 137, 138, 139. Pl. XVI.) As soon as the cambiumcells come to a period of comparative rest, they contain alcaloid. Those which afterwards differentiate to cells of the medullary rays, plates or bastparenchyma collect more and more alcaloid until they contain large quantities of it. Sieve-tubes, conducting cells and bastfibres contain no alcaloid. This explains why the outer layers of „Cinchonabark" contain more alcaloid than the inner ones do. The vulgus „Cinchonabark" of course consists of secundary bark plus primary bark plus corklayers. The primary bark contains, as we saw allready, alcaloid in all cells except in those which contain oxalic acid and in the „Gummiharzschlaüche"

The secundary bark on the contrary, consists of parenchyma containing alcaloid, of barkfibres and sieve-tubes containing none, while the number of sieve-tubes increases the nearer one comes to the cambium. By the continuous originating of new layers between wood and bark, the peripheral sieve-tubes become more and more compressed so that finally the most external ones become unrecognisable and hardly occupy any room. If every parenchyma cell contains about the same quantity of alcaloid it stands to reason that the secondary part of the bark must contain less alcaloid than the outer one, as in the secondary part there is a large tissue, *without* alcaloid: the sieve-tubes, while no such tissue exsists in the primary part. This result is confirmed by analysis. Broughton found the following quantities:

Cinchona succirubra.

Part belonging to the secondary bark 5. 94%
„ „ „ primary „ ..... 7. 98 „

But not only this; on the grounds above mentioned, the

inner layers of the secondary bark must be poorest, the outer ones richer and the primary bark covering them the richest in alcaloid. This also is confirmed by the chemical analysis.
Moens gives the following as results of his analyses:

Cinchona Calisaya.

Part belonging to primary bark	5. 6%
outer half of „ „ sec. bark	5. 36"
inner „ „ „ „ sec. „	2. 71"

2c. *Tissues formed by the phellogen.*

The corkforming tissue arises from the subepidermal cell-layer. When the cells of this layer are beginning to divide the amount of alcaloid in them decreases, until the new cambium contains no alcaloid at all (fig. 136. Pl. XVI).

The phellodermcells formed by the cambium soon after their originating contain alcaloid, while as a rule a longer time must elapse before the formed corkcells contain any (fig. 137 Pl. XVII); yet exceptions occur as is seen from the three cells in the middle of fig. 137, where the young corkcell, the cambium cell and the young phelloderm cell all three contain alcaloid. Somewhat older, nucleated, living corkcells contain considerable quantities of alcaloid (c. fig. 133, 134, Pl. XV. fig. 139 Pl. XVI).

The filling-up tissue of the lenticells is conspicuous by its comparatively large amount of alcaloid yet the underlying phellodermcells contain even more; (c. fig. 138 Pl. XVI). old, dead, corkcells contain no alcaloid. (1)

In those parts of the primary and secondary bark which

(1) At least not in their lumen, possibly their membranes are impregnated with it but this can not be shown microchemically.

are cut off from further nourishment by slanting corklayers, alcaloid is met with occasionally, but always in small quantities.

c. *The Root.*

If one brings a longitudinal section of a roottip in the iodinesolution the meristema stains a dark brown at once. It looks exactly as if much alcaloid were present in it. A contrôle-experiment however shows this colour to be due to albuminous substances and not to alcaloids.

The rootcap contains no alcaloid as little as do the epidermis or the roothairs. Yet one can obtain sections in which the peripheral celllayer contains alcaloid (fig. 143 Pl. XVII, fig. 145 Pl. XVIII). In these sections the epidermis has allready been thrown off; the peripheral layer consequently is no epiderm, but the formerly subepidermal layer now called exoderm. Even on a much younger stage this subepidermal layer contains alcaloid (fig. 144 Pl. XVIII, fig. 140 Pl. XVII, fig. 147 Pl. XVIII). This subepidermal layer may contain alcaloid up to a point very near to the tip of the root, or the alcaloid may begin at a much further distance from the latter; roots which are in a comparative period of rest apparently have the alcaloid very close to the meristemal tip, those growing rapidly not in so young a part.

The primary rootbark contains no alcaloid, nor does the central cylinder, besides in the exoderm it is found in such young roots in the endoderm (fig. 141, 142 Pl. XVII) or and this is more generally the case in a layer immediately outside of the endoderm (fig. 140. Pl. XVII). In a somewhat older root, beginning to throw of its epiderm (fig. 141 Pl. XVII) some little alcaloid appears in some of the primary bark cells, which later on may increase yet (fig. 145 Pl. XVIII). In the centralcylinder no alcaloid is found neither in the parenchyma nor in cambium or pericambium (pericycle).

As soon as the pericycle begins to form phellogen, this phellogen and the young corkcells formed by it or the latter only, may contain alcaloid; (fig. 143 Pl. XVII at x). As is known the action of the phellogen throws of the primary rootbark (fig. 143 Pl. XVII.). In the cells of the primary root Janse's endophyte is found.

After throwing of the primary bark nothing remains outside of the central cilinder but the secondary bark, which even on a very young stage contains some alcaloid.

Later on this rootbark resembles the stembark greatly, only no primary bark is found on its exterior side. The alcaloid is here localised in exactly the same manner as in the stembark. (comp. fig. 130 with fig. 131 Pl. XIV and fig. 146 Pl. XVIII).

The secondary wood of the root contains alcaloid as does the stemwood in the medullary rays, its prolongations and woodparenchyma.

d. *The organs of Reproduction.*

In meristematical condition the different parts of the flower contain no alcaloid. In the same way as in the leaves the alcaloid appears gradually in corolla and calyx. Here also a maximum is met with at an early age. Adult petals and sepals however contain more alcaloid in each cell than do adult vegetative leaves.

The epiderm of the calyx contains no alcaloid, the supepidermal layer contains more alcaloid than any other one. It can be said that in a general way the external parenchyma contains more alcaloid than the internal one, this differs however in different flowers. Fig. 149. Pl. XIX pictures a fair average. The corollar leaves contain no alcaloid in the epiderm and in them also the external parenchyma contains more alcaloid than the internal one does. At this stage the stamens show alcaloid in the connective

only. Later on this changes; small quantities appear in the three layers of the walls of the pollenchambers (c. fig. 150 Pl. XIX). Consequently the alcaloid is here met with in the epiderm also.

With increasing age the internal one of these three layers degenerates and now the two outer layers alone contain alcaloid (fig. 151. Pl. XIX.) In adult pollenchambers the epiderm alone contains alcaloid (fig. 152 pl. XIX). Archesporium and tapetum contain no alcaloid, nor does the adult pollen.

The gynaeceum contains alcaloid in the parenchyma cells of the pistil (fig. 149 Pl. XIX), the wall of the fruitprimordium contains alcaloid also, which alcaloid is for the greater part situated towards the exterior, while the epiderm remains deprived of it. (fig. 155. Pl. XIX, fig. 156 Pl. XX).

From the very beginning (c. fig. 148. Pl. XVIII) the placenta and the internal layer or horny wall of the fruit are deprived of alcaloid.

This horny layer increases in thickness out of all proportion to the increase of the other parts so that the percentage of alcaloid decreases with age in the fruit. Later on the alcaloid disappears from the parenchyma outside of the horny layer so that the dry fruit contains none or but very little alcaloid. The ovules also are always deprived of alcaloid (c. fig. 155, 156, Pl. XIX and XX). The central partition of the fruit contains but very little alcaloid. The peduncle of the flower and that of the young fruit contain alcaloid in the parenchyma not in the sieve-tubes (fig. 155 Pl. XIX fig. 156, Pl. XX).

The iodine solution however precipitates in the placenta and in the epidermis of the ovules a substance insoluble in alcohol and consequently no alcaloid (c. fig. 159 Pl. XX). The xanthoprotein-reaction shows it to be a mixture of albuminous substances and some gum (c. fig. 158 Pl. XX).

In the seed, I have been unable to demonstrate the presence of an alcaloid either in the embryo, or in the endosperm. The precipitates obtained by the aid of iodine (fig. 154 Pl. XIX fig. 157, 160 Pl. XX) are due to the presence of albuminous substances, as is shown by the xanthoprotein-reaction (fig 162)

Owing to the large quantities of albuminous substances here present, small quantities of alcaloid may have escaped detection.

As soon as the cotyledons of the germinating seeds have formed chlorophyll, they form alcaloid also (fig. 161 Pl.XX).

f. *General remarks about the localisation of the alcaloid.*

The most important result of the investigation mentioned above for physiological experiments to follow is the fact that no alcaloid is found in the sieve-tubes, in other words not in that tissue which preeminently serves to transport the albuminous substances. On the contrary it is found in the parenchyma, the tissue which is especially adapted to the transportation of carbohydrates. It is also found in the assimilatory tissue, while it is not present as a reserve substance in the seeds.

The alcaloid is furthermore present as the content of living cells only, though in rare cases *perhaps* it is present impregnating the membranes of dead cells f. e. in the pith of the stem, old woodfibres, old corkcells. But even if it be there; which is by no means proved, this is a secondary phenomenon arisen by the cellsap diffusing towards the outside on the death of the cell. In the bastfibres no al caloid is present.

Normally alcaloid is consequently present exclusively as the content of living parenchyma-cells or of other cells differing but little from parenchyma.

I believe that every parenchyma-cell may contain alcaloid at some time or other except those which contain oxa-

lic acid. I never saw oxalic acid (as oxalate of lime) and alcaloid in the same cell.

Generally speaking the alcaloid is dissolved in the cellsap in young organs viz: leafstalks, leafparenchyma near the growing points, young bark; as an amorphous solid in the old parts like the cells of the secondary bark.

Frequently it is present as a tannate, wether occasionaly as an other salt has not been investigated.

Besides in the bark, much alcaloid is present in very young organs, near the stem-growing point, young but not too young leaves etc.

Very active organs undergoing many and rapid divisions apparently contain no alcaloid, f. e. it is not found in the *very active* part of the stem-growing point, in the cambium, in the active part of the root-tip.

Quite close to the stem-growing point considerable more alcaloid is found than quite close to the rootgrowing point.

Part II. Where is the alcaloid formed?

As is well known all the starch present in the bark of trees is formed in the leaves and transported towards the bark in small quantities. The albuminous substances also, most probably at least, are formed in the leaves. Where the leaves are the originators of such important substances it was highly suggestive to investigate wether they formed the alcaloid also. The published chemical analyses gave much cause to inquire into a possible alcaloid-forming property of the leaves in as much as the results of these analyses are so different that a great inconstancy in the quantity of alcaloid present seems to exist. It was consequently to expect that this inconstancy of the leaves in regard to the quantity of alcaloid present would be due to a temporary transportation of alcaloid towards the stem.

How large the differences between the analyses of various authors are, results from the following quotations! In 1869 we find in Howard: (1)

I obtained from these leaves (dry ones sent to him from India) to the extent of 0. 11 % of alcaloid. From these data it seems to follow that the leaves (C. succirubra) will not supply a material for the extraction of Quinine but that they will nevertheless be very usefull when used fresh or in recently prepared decoction or infusion for the cures of the fevers of the country.

He furthermore cites from a report (2):

„I regret to be obliged to confirm the opinion I expressed in my last, that the leaves will not supply material for the extraction of quinine, although the quantity of the first rough precipitate from an acid solution having the appearance of a hydrated alcaloid is considerable more than I succeeded obtaining before, being equal to 1, 31% of the weight of the leaves....Nevertheless the further prosecution of the inquiry and the attempt to purify the alcaloid, showed me clearly that I had to do with a state of things *very different from that which existed in the bark* and that I should not succeed in obtaining an available salt of quinine.

Later on Howard apparently found even less. Moens (3) quotes from an article by Howard (Ph. I. F. Jan. 1873 p. 541) which is inaccessible to me, that Howard once found a little; but later obtained no alcaloid at all from twenty pounds of leaves.

Broughton obtained from fresh leaves of C. succirubra also only 0. 0041 % of alcaloid; *0. 0016 % of which was quinine*,

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(1) Howard. The Quinology of the East-Indian plantations. Reeve & Co. Covent Garden. 1869 p. 14.

(2) l. c. 15

(3) Moens. De Kinacultuur in Azie p. 301.

from dry leaves 0.019 % of alcaloid; 0.008 % of which was quinine, and Moens obtained from Ledgeriana leaves not more than traces. In fresh leaves of C. officinalis Broughton (1) found 0.0035 % of alcaloid; 0.0015 of *which was quinine*, while de Vry found no alcaloid in leaves of C. Calisaya.

In 1896 de Vry (2) found in dry leaves of C. C. Ledgeriana sent to him from Java by Mr. van Leersum 0.162 % of amorphous alcaloid. *Crystalised* alcaloids were not found.

Method.

The first thing to do was to find a method adapted to our purpose. *If possible this method should allow the detection of the alcaloid in one half of a leaf.*

This requirement is essential for physiological purposes as only by such a method it becomes possible to *examine the same leaf at two different moments.* In this way we can obtain a degree of exactness, which by no other means can be reached. *Two leaves apparently absolutely the same* can show great differences, while the one is full of alcaloid the other one may be empty. If now one picks the empty leaf in the morning and the full one at night, one would suppose the alcaloid found in the latter to have been formed during the day, while in fact it was allready present in the morning. The method employed, an adaptation of the general method for the discovery of the alcaloids, is thus:

Throughout the investigation the two halves of the same leaf were used. These halves were always longitudinal ones. They were obtained by cutting exactly along the midrib of the leaf. In this way the leaf was divided in two unequal parts one part containing the midrib, the other not.

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(1) Blue book, 1870 p. 238.

(2) de Vry. Kinologische Studiën, Nederl. Tydschr. v. Pharmacie etc. 1896 p. 104

The piece without the midrib was examined at once, that containing the midrib was used for the experiment. It remained on the tree, was put in water, laid on moist blotting paper or treated in other ways. At the end of the experiment the midrib was cut off and the remaining half of the leaf examined.

In this way the leaf-parts to the right and the left of the same midrib were always compared with each other.

For examination the leaf-parts were cut into very small quadrates and boiled in alcohol containing $^1/_2$ % of HCl (20 cc. conc. HCl on the Liter) for an hour. This took place on the waterbath in Erlenmeyer-bottles, stoppered by a cork into which a long glasstube, serving as a cooler, was put. The alcohol was afterwards poured into porcelain dishes, placed on the waterbath and the whole evaporated until nearly dry. Afterwards the dishes were filled up with water and evaporated again until nearly dry also, so as to be sure of the total escape of the alcohol. After this water was added, again filtered and the filtrate collected in a separotory. After adding caustic potash until alkaline solution it is shaken with chloroform, the chloroform collected in a watchglass, put on the waterbath and all chlorofrom evaporated. The residu is dissolved in water, containing $^1/_2$ % of HCl (20 cc. conc. HCl to the Liter.) By strong rubbing the resinous substances, sticking to the watchglass are mixed with this solution, the whole filtered and the filtrate used for the alcaloid tests. I followed the chemical part of this method owing to the advice, kindly given by Dr. W. G. Bjorsma to whom my sincere thanks are here offered.

At the commencement of this investigation nearly all the usual alcaloid reagents were used. When alcaloid was present they all gave sumptuous precipitates. To decide wether the leaves were empty or not these reagents frequently were *too* sensitive, even the very smallest quan-

tities causing the appearence of a precipitate. It was consequently decided to use a less excessively sensitive reagent, yet a more than sufficient sensitive one, viz. caustic potash and to consider a leaf, which on application of this KOH gave no precipitate to be empty, one which gave one as to be „*full*". Using this reagent one can estimate quantities to a certain degree f. e. traces, very little, but little, pretty much, much, very much and an exceedingly large quantity, but to be safe, only those leaves were used for the experiments as to the formation of alcaloid, which gave no reaction with KOH.

Several preliminary experiments had taught me that the two halves of a leaf, examined at the same moment gave corresponding results. As an example, I can state the results obtained with leaf-halves, from which I did not previously know which two belonged together.

1	halves belonging to leaf N°.	1.—	but little
2			„ „
3		2.—	pretty much
4	„ „ „ „		„ „
5		3.—	very much
6	„ „ „ „		„ „
7		4.—	traces
8	„ „ „ „		traces
9		5.—	empty
10	„ „ „ „		„

Yet these discriminations were not used, as allready stated above.

In the statements below the expression „empty" means that the chloroform residu dissolved in water acidulated with HCl gave no precipitate with KOH, while „full" means that KOH caused a considerable precipitate.

It was necessary to first inquire into the quantity of alcaloid present in the leaves of Cinchona.

At a former occasion I called attention to the fact that quantities expressed in percentages of the dry matter are no measure for the absolute quantity present in an organ.

If we suppose that a young leaf of C. Succirubra contains 1% of alcaloid and an adult one of the same tree 0.1% it remains possible yet that the adult leaf contains more alcaloid than the young one.

For example: The dry weight of a young leaf of C. Succirubra was found to be 250 milligrams, while that of an adult one of the same tree amounted to 3 gram or 3000 milligram.

A young leaf containing 1% of alcaloid consequently contains 2,5 milligram, an old one of but $^1/_{10}$ % of alcaloid contains 3 milligram.

Or, although the percentage of alcaloid contained in an adult leaf is but one tenth of that in a young leaf, yet more alcacaloid is present in an adult than in a young leaf.

Now let us calculate the quantity of alcaloid to be delivered daily by the leaves necessary to supply the quantity present in the bark!

An adult tree of C. Succirubra contains about 700 gram. of alcaloid in the bark as is clear from the following calculation.

Moens (1) gives for the production of C. S. trees of 9 years old, under favorable conditions 9.38 KG. of dry bark, 6.92 KG. of which were stembark—1 KG. bark of branches and 1.46 KG. of rootbark.

This is certainly a fine production for trees of 9 years of age as others mention for 8 year old trees 3 KG.

After Moens (2):

C. Succirubra.:	Stembark 1st. Kind contains	7.7%	of alcaloid
	bark of the branches	3.5	„ „
	rootbark	9.1	„ „

(1) l. c. p. 226
(2) l. c. p 270/71

or, a C. S. of 9 years contains in the stembark 532. 84 gr.

in the branches	35. „
in the root	132. 86 „
Total	700. 70 „

Consequently we get this calculation:

Total alcaloid produced in 9 years:

700. 70 gr=700700 milligram

or for every day an average of

$$\frac{700700}{3285} = \pm 210 \text{ milligram.}$$

Accepting 3 grams to be the weight of adult C. S. leaves and them to contain $^1/_{10}$ of a percent of alcaloid, every leaf contains 3 milligram or 70 (seventy) leaves would be sufficient to produce the quantity of alcaloid present in the bark of C. S. provided they transport every day the quantity of alcaloid present in them.

On a very poor, weak C. S. of about 6 ycars on Tjinjiroean I counted 781 leaves, on a well developped tree estimated at 12 years 3155 leaves. This last tree consequently, if emptying its leaves every day, would be able to form 3.5 KG. of alcaloid a year.

On a tree of C. Ledgeriana of Tirtasari I counted 10971 leaves and found for the average weight of a dry leaf somewhat more than 0,5 gr. Accepting 10.000 to be the number of leaves, the dry weight of one leaf being 0.5 gr., we obtain for that tree 5000 gr. of leaf weight. Accepting quantity of alcaloid present in the leaves to be $^1/_{10}$ (1) of the a percent, we see that 5 grams of alcaloid could be

(1) De Vry found 0.192 % consequently *considerably* more. Besides it is well to bear in mind that the quantity of alcaloid found in a leaf signifies only the remnant remaining at that particular moment, while a continuous transportation takes place. Consequently a leaf containing at a certain moment 3 milligrams of alcaloid may have transported considerably more than 3 milligrams towards the stem that day.

transported towards the stem every day, or in a year nearly 2 KG.

As we know such quantities of alcaloid are never acumulated in the bark.

We have thus come to the following conclusion:

The quantity of alcaloid present in the leaves of a C. S. and of a C. L. is, provided it can be transported towards the stem every day, many times sufficient to account for the quantity of alcaloid found in the bark or in other words the leaves would be able to form this quantity of alcaloid.

It is now asked *is* a leaf of C. S. able to transport towards the stem inside of 24 hours the quantity of alcaloid present in it?

From my series of experiments I quote the following:

6 p. m. Sept. 18. 1899		6 a. m. Sept. 19. 1899
No. 284	full	empty
285	„	„
286	„	„
287	„	„
288	„	„
289	„	„
291	„	„
292	„	„
6 a. m. Sept. 21. 99		6 p. m. Sept. 21. 99
305	full	empty
308	„	„
310	„	„

Or, leaves of C. S. are able to get rid of all their alcaloid inside of twelve hours.

We have allready seen that a tree of C. L. with 10.000 leaves would be able to form in this way 2 KG. of alcaloid a year. Why don't we find that then?

Several reasons can cause this: f. e. changing of the alcaloid to another substance, but one of the reasons is that

the leaves are not always empty in the morning f. e.

On the 21 th. of Sept. 1899 it was found at 6 a. m. that:

No. 304 contained exceedingly large quantities of alcaloid
305 „ „ „ „ „
306 „ very little „ „
307 „ „ „ „ „
308 „ much „ „ „
309 „ exceedingly „ „
310 „ pretty much „ „
311 was empty „ „ „
312 contained exceedingly „ „
313 „ „ „ „ „

In the preceeding night consequently but one of ten leaves become emptied.

Next day the result was somewhat better, yet many leaves remained full.

On the 22 nd. of Sept. 1899 was found at 6 a. m.

No. 314 much
315 nothing
316 „
317 little
318 very much
319 little
320 traces
321 nothing
322 pretty much
323 exceedingly large quantities.

Consequently 3 out of ten leaves were empty. This of course can have several reasons, one I believe to have found in the considerable degree of coldness which can be reached here at night. (1)

(1) The garden is situated at a height of 4200 feet.

The 2nd. of Oct. for example I found on a young tree having passed the night outside:

No. 351 much
352 empty
353 much
354 empty
355 empty
356 much
357 very much
358 much
359 exceedingly large quantity
360 much.

Out of 10 leaves consequently but 3 were empty.

Next day, the 3d. of October another small tree, which served for the experiment, was put in the glasshouse for the night and was examined with the following result:

Oct. 3. 6 p. m.				Oct. 4. 6. a. m after night in glasshouse:
361	exceedingly	large	quantities	empty
362	„	„	„	„
363	„	„	„	„
364	„	„	„	„
365	„	„	„	„ [1]
367	„	„	„	empty
368	exceedingly	large	queantities	„ [1]
369	pretty much	„	„	empty
370	„	„	„	„

Consequently out of *10 leaves all ten were empty*

Next day also, with a plant put in the dark room, a favorable result was obtained:

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[1] Even the picric-acid reaction failed.

Oct. 4. 6. p. m.		Oct. 5. 8. a. m.
No. 371.	so large a quantity as never met with before	empty
372	much	„
373	pretty much	„
374	„ „	„
375	much „	„
376	„ „	„
377	little „	„
378	pretty much	„
379	nothing „	„
380	nothing „	„

Next day two leaves (1) gave the same result:

Oct. 5. 6. p. m.		Oct. 6. 6. a. m.
No. 383	pretty much	empty (2)
388	very much	empty (2)

The fact, that the theoretically possible quantitiy is not transported by the leaves is furthermore due to the circumstance that apparently they do'nt make that quantity every day. At the end of a very foggy and rainy week I found at 6. p.m. Sept. 29:

No. 342	traces
343	nothing
344	little
345	nothing
346	nothing
347	traces
348	very much
349	exceedingly large quantity
350	little
350 a.	little

(1) The other 8 leaves of this series did not form any alcaloid the previous day.
(2) Even picric acid failed to cause a precipitate.

While at the end of a clear day the following was found:

Oct. 3. 6. p. m.

361 exceedingly large quantity
362 „ „ „
363 „ „ „
364 „ „ „
365 „ „ „
366 traces
367 „
368 exceedingly „ „
369 pretty much
370 „ „

By which we may conclude that climatological influences are felt in the formation of the alcaloid.

We have thus seen that Cinchona leaves at one time do contain alcaloid while at another they do not, the question is now what becomes of this alcaloid, is it transported towards the stem or is it used by the leaves themselves?

To decide this experiments with cut leaves are necessary.

When the leaf itself uses the alcaloid, it should disappear under favorable circumstances inside of a comparatively short time. We will see that such does not happen as becomes clear on perusal of the following tables.

In the first place the influence of darkness was studied.

As is seen from a look at Tabula I, no effect whatever was caused by it.

An addition of glucose to the water, could not induce the leaves to part with their alcaloid (T. I)

A sejourn in the light, be it with the leafstalk immersed in water, or the whole leaf placed on moist blotting paper inside of a Petri-dish did not lead to the using up of the alcaloid (c. T. II.).

Tabula I.

Number.	AFTER HAVING BEEN IN THE DARK FOR:												
	6 days.	7 days.	8 days.	9 days.	10 days.	12 days.	13 days.	14 days.	15 days.	22 days.	27 days.	36 days.	37 days.
No. 1	full												
- 3	full												
- 4	full												
- 11								full					
- 12								full					
- 15				full									
- 17				full									
- 20				full									
- 21				full									
- 23				full									
- 25								full					
- 26								full					
- 27						full							
- 28						full							
- 29						full							
- 30									full				
- 31									full				
- 32				full									
- 33				full									
- 34									full				
- 35									full				
- 36						full							
- 37							full						
- 38							full						
- 40									full				
- 41							full						
- 42							full						
- 43						full							
- 44						full							
+ 5 % glucose 45.		full											
- - 46.			full										
- - 47.			full										
- - 48.			full										
- - 49.			full										
- - 50.					full								
- - 51.					full								
- - 52.					full								
- - 53.					full								
- - 54.					full								
- - 55.					full				full				
No. 56					full				full				
- 57					full								
- 58					full				full				
- 59					full				full				
- 75											full		
- 76											full		
- 77											full		
- 78											full		
- 79											full		
- 80											full		
- 81											full		
+ 5 % glucose 89.										full			
No 189												full	
- 206													full
- 207													full
- 209													full

Tabula II.

Number.	Treatement	CUT LEAVES IN THE LIGHT AFTER:					
		7 days	11 days	14 days	16 days	17 days	36 days
No. 91	On blotting paper in petri dishes	full					
„ 92	„	full					
„ 93	„	full					
„ 94	„	full					
„ 95	„	full					
„ 96	„	. . .	. . .	full			
„ 97	„	. . .	. . .	full			
„ 98	„	. . .	. . .	full			
„ 100	„	. . .	. . .	full			
„ 101	„	. . .	. . .	. . .	. . .	full	
„ 102	„	. . .	. . .	. . .	. . .	full	
„ 103	„	. . .	. . .	. . .	. . .	full	
„ 104	„	. . .	. . .	. . .	. . .	full	
„ 110	„	. . .	. . .	. . .	. . .	full	
„ 114	in water	. . .	. . .	full			
„ 115	„ „	. . .	full				
„ 116	„ „	. . .	full				
„ 119	„ „	. . .	. . .	full			
„ 120	„ „	. . .	. . .	full			
„ 121	„ „	. . .	full				
„ 132	„ „	. . .	. . .	. . .	full		
„ 133	„ „	. . .	. . .	. . .	. . .	. . .	full
„ 134	„ „	. . .	. . .	. . .	full		
„ 135	„ „	. . .	. . .	. .	full		
„ 136	„ „	. . .	. . .	. . .	full		
„ 179	„ „	full					
„ 181	„ „	full					
„ 185	„ „	full					
„ 186	„ „	full					

We have consequently thus far seen that Cinchona leaves contain alcaloid, which alcaloid disappears inside of 12 hours from the leaf attached to the stem, while in cut leaves it remains even after several weeks.

Now the question presents itself, wether empty leaves are able to form alcaloid again, inside of a short period.

That leaves connected with the stem are able to do so is proved by the next experiments:

Sept. 20. 6 a. m		Sept. 20 6. p. m.
294	empty	full
295	„	„
296	„	„
297	„	„
298	„	„
299	traces	„
300	„	„
301	„	„
302	„	„
311	traces	„

In these cases the possibility remains that the originally empty leaves got the alcaloid lateron found in them from the alcaloid present in the bark.

It was therefore necessary to demonstrate that *cut* empty leaves, were able to form alcaloid if brought under favorable conditions.

The next experiments show that such can be done.

Half of leaf examined		Corresponding half in H_2O + $^1/_4$ % NH_4 until *July 17.*
	July 11.	
No. 141	empty	full
142	empty	full
145	empty	full
	July 17.	*July 24.*
172	empty	full
173	empty	full

	Sept. 4.	$^1/_{10}$ % NH_4 *Sept. 11.*
217	empty	full
218	empty	full
219	empty	full
	Sept 6.	*Sept 12.*
226	empty	full
227	„	„
228	„	„
230	„	„
231	„	„
232	„	„
	Sept 7.	*Sept 12.*
234	empty	full
235	„	„
236	„	„
237	„	„
238	„	„
242	„	„
243	„	„
Cinchona calopteraSept 8.		in river water until Sept. 13.
244	empty	full
247	empty	full

Resuming:

I. Leaves of C. Succirubra and those of C. Ledgeriana contain a many times sufficient quantity of alcaloid to account for all the alcaloid present in the bark supposing the leaves to be able to transport their alcaloid towards the bark once in twentyfour hours.

II. A full leaf of C. Succirubra can empty itself inside of twelve hours.

III. The disappearance of this alcaloid is not due to it having been used up by the leaf; the cut leaf is not able to dispose of it, even if in stead of twelf hours one allows 36 days for this process.

IV. An empty leaf of C. Succirubra connected with the mo-

therplant is able to form its alcaloid anew inside of twelve hours.

V. Empty cut leaves also are able to form alcaloid inside of a few days at least.

It will therefore be allowed to draw these conclusions:

A. The disappearance of the alcaloid from the leaves is due to transportation towards the stem.

B. The alcaloid later on found in a previously empty leaf, has been made by that leaf itself.

Consequently: The alcaloid present in the bark of Cinchona has been formed in the leaves, transported in small quantities towards the stem and there stored away.

From the microchemical investigation we know that it is transported as a fluid, stored up as an amorphous solid.

We know from analyses made by BROUGHTON that the leaves of Cinchona Succirubra contain quinine besides the other alcaloids, so that transportation of that substance would account for the quinine present in the bark.

Yet we know that transformation of the alcaloid must take place in the bark itself in as much as DE VRY and BEHRENS have found that the leaves of C. C. Ledgeriana contain no crystallisable alcaloids or i. o. w. no quinine, while the bark contains such in large quantity. The leaves of C. Ledgeriana contain nothing but amorphous alcaloid and consequently we have to accept a transformation of amorphous alcaloid to crystallisable ones.

A transformation from an alcaloid to another is by no means inconceivable as quinine is known to be a cinchonine derivative, through substitution of the groupe CH_3O for a CH groupe. The chemical name of quinine is paramethoxycinchonine.

But even more, GRIMAUX and ARNAUD (1) *have made* qui-

(1) C. R. 122. p. 774; 114, p 672

nine from cupreine. Cupreine is an alcaloid found in the bark of Remija pedunculata FLUECK.

It has consequently been *proved* that alcaloids found in different *genera* of plants can even outside of those plants been transformed to each other, why should not a plant be able to transform alcaloids formed by itself?

Some occasional experiments with strychnos-species have given me indications of such a transition from strychnine to brucine in the strychnos leaves.

Therefore to conclude, it may be admitted that: *Cinchona trees form their alcaloid in the leaves, transport it to the bark where it is stored either in its original form or after having been changed to another alcaloid.*

Such transitions from one substance to another are by no means rarely met with in plants physiology, it only needs to be reminded of the behaviour of starch and sugar.

It is self-evident that these experiments do'nt exclude the possibility of a formation of alcaloids in the bark itself, yet it seems to me that these experiments together with the reasoning stated above, make it plausible that anyhow this will be of very much less importance than that formed in the leaves.

Of the previously published analyses a series by Mr. v. Leersum showing that trees with a yellowish foliage contain a lesser percentage of alcaloid than those with a dark-green one is of course much in favour of our theory.

These experiments show that it is of prime importance for the Cinchona-planter to do all in his power to obtain a rich foliage on his trees, a proceeding which in the last years has been followed in the Cinchona-plantations of the Dutch Government.

It may not be devoid of interest to state in what direction the author thinks further experiments will have to proceed.

Without any doubt the first step is to examine the way in which the alcaloids originate in the leaves, wether and which simpler substances are used for the building up of the alcaloid-molecule or wether the alcaloids are decomposition products of higher bodies f. e. of proteids.

The first question arising is wether a synthesis of alcaloid by the plant comes within the range of possibility. Genuine alcaloids are bodies (1) containing a pyridin nucleus, they consequently belong to the groupe of the pyridin derivatives.

The first question to be answered is consequently: is the plant *theoretically* able to form a pyridin nucleus. Now according to PICTET (2) pyridons (lower pyridin-derivatives) can be formed from pyrons and ammoniac at the ordinary temperature. Pyron derivatives now occur in the plants f. e. meconic acid in Papaver somniferum, and chelidonic acid in Chelidonium majus and Helleborus alba.

Another pyron derivative is cumalic acid; this, it is true has not been demonstrated in the plant but as it can easily be made from mallic acid (3) one of the most common plants-acids, this is no objection for our purpose. Even if it has not been overlooked in the plant it can be present as a transitory condition only, arising during chemical transformations taking place in the plant.

We can therefore say that a synthetical formation of pyridin-derivatives from mallic acid and ammoniac does not belong to those processes which a priori must be considered impossible for the plant.

It could be done in this way.

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(1) For particulars I must refer the reader to the Dutch text.

(2) La structure chimique des alcaloides végétales.

(3) Vide. RICHTER. Org. Chemie, 5th Edition 1888 p. 537. Of course as mallic acid contains but 4 C atoms not arranged in a ring, at least 2 molecules are necessary for this. See further Berl. Ber. 17 p. 936 and 2385.

Two molecules of mallic acid are changed by dehydration etc. to cumalic acid, by means of ammoniac this is changed to pyridon carbonic acid (a pyridin-derivative).

Through further changes of this body allready containing a pyridin nucleus the higher pyridin-derivatives, the alcaloids could be formed.

It is consequently not at all impossible that the alcaloids are formed by direct synthesis and not as decomposition products of proteids.

As our experiments have shown that leaves having ammonia (as NH_4Cl) to their disposition can form alcaloid it is of importance to find out whether the ammonia plays a rôle there as can be ascribed to it according to the theoretical considerations mentioned before.

How does this concern the Cinchona alcaloids? These are derivatives of higher bodies of the pyridin-series. As pyridin is the nucleus of the alcaloids considered above, so quinoleine (synonymous with leucol, leucoline and quinoline) is the nucleus of the Cinchona alcaloids. While pyridin contains but one benzine ring, quinoleine contains two. That such bodies with two benzine rings can be obtained from bodies containing but one is seen from the fact that quinoleine has been obtained from cinnamomic acid a body containing but one benzine nucleus.

In the most different parts of the Cinchona trees we find an acid containing a benzine ring, it is called cinchona acid (Kinasäure).

It is consequently not at all impossible that cinchona acid by means of an ammonia-derivative could be changed to quinoleine.

From this quinoleine γ phenylquinoleine can be derived, which after Konigs (v. Pictet. p. 94) can be considered as the mother substance of the Cinchona alcaloids.

A large distance yet separates the alcaloids from this

phenylquinoleine; we wo'nt go into that as it is not our object to go into detail of the structural formula of Cinchona alcaloids.

All I wished to show here is that it is not at all impossible that plants acids play a considerable rôle in the formation of alcaloids.

Although all this latter part as far as it concerns the plant is speculative, all what concerns the constitution of these bodies is based on really obtained results.

I therefore do'nt hesitate to state that the results obtained along the line of purely chemical investigation compel us to find out which rôle the plants acids play in the formation of the alcaloids.

It is along this line that investigations will be continued.

Mountaingardens of 's Lands Plantentuin.
Tjibodas. Oct. 99

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(1) vide: PICTET p. 81
(2) MOENS l. c.

ERRATA.

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